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Anatomy of vegetative organs of *Scutellaria agrestis*, a medicinal plant cultivated by riverine populations of the Brazilian Amazon

Andréia B. de Oliveira,[1] Maria S. de Mendonça,[1] Renata M. S. A. Meira*[,2]

[1]Laboratório de Botânica Agroflorestal, Universidade Federal do Amazonas, Brazil,
[2]Laboratorio de Anatomia Vegetal, Departamento de Biologia Vegetal, Centro de Ciências Biológicas, Universidade Federal de Viçosa, Brazil.

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Abstract: *Scutellaria agrestis* A. St.-Hil. ex Benth., Lamiaceae (trevo-roxo) is cultivated for medicinal purposes by residents of the riverine communities in the State of Amazonas, Brazil. This study aimed to characterize the anatomy and to make histochemical analysis on vegetative organs of *S. agrestis*. Samples of the leaf, stem and root were collected from five plants cultivated by the communities located in the Solimões river, Amazon. These samples were fixed and prepared following standard techniques for scanning electron microscopy and for light microscopy. Histochemical tests were carried out on sections to detect the main classes of compounds present in the secretion. Numerous glandular trichomes are seen in both leaf and stem of *S. agrestis*. The leaves are amphi-hypostomatics and show dorsiventral mesophyll. Hydathodes are present at the tip of the marginal teeth. Anthocyanin pigments occur into the epidermal cells of the stem, petiole, and abaxial leaf surface. The petiole is concave-convex shaped and bears collateral vascular bundles. The stem showed square-shaped, evident endoderm, collateral vascular bundles and parenchymatous pith. The root displays a typical protostelic structure. Idioblasts containing mucilage and phenolic compounds occur in the cortex. These data are important, as they can be useful to identify this species, contributing to the quality control of the medicinal plant.

Introduction

Lamiaceae is a large family which comprises 252 genera and 6800 species (Judd et al., 2009) and in the Brazil, there are 34 genera and 498 species (Harley et al., 2013).The species grow in all types of environments, at various altitudes, displaying a cosmopolitan distribution. They are characterized by opposed or verticillate leaves, indented or serrated margins with squared branches and stem; glandular hairs with aromatic oils (including terpenoids) and non-glandular, small flowers arranged in crests (Ribeiro et al., 1999; Souza & Lorenzi, 2008; Judd et al., 2009). *Scutellaria* L. is a genus of Lamiaceae comprised by almost 300 species (Paton, 1990; Pool, 2006; Judd et al., 2009). This species is found mainly in temperate regions and tropical mountains, including in Europe, North America and East Asia (Bruno et al., 2002), with some species reported in Central America, Colombia, and Equador (Alonso, 1990; Pool, 2006). In the Brazil four species are endemics: *S. alborosea* Lem., *S. leucantha* Loes., *S. tubiflor*a Benth. and *S. uliginosa* A.St.-Hil. ex Benth., however five other species have also been reported: *S. agrestis, S. incarnata* Vent., *S. platensis* Speg., *S. purpurascen* Sw., *S. racemosa* Pers. (Alonso, 1990; Almeida & Albuquerque, 2002; Cassino, 2010; Harley et al., 2010; 2013).

Many species of *Scutellaria* (*S. baiacalensis* Georgi, *S. amoena* C. H. Wright, *S. linearis* Benth., *S. viscidula* Bunge, *S. strigillosa* Hemsl, *S. prostrata* L., *S. grossa* Wall, *S. barbata* D. Don, *S. hypericifolia* H. Lév, *S. gallericulata* L., *S. discolor* Wallich ex Benth., Pl. Asiat. Rar., *S. seleriana* Loes, *S. ovata* Hill, among others) have long been used in traditional medicine by people of Asia, Europe and America (Shang et al., 2010). In recent years, these species have been studied in the fields of health, chemistry and phytochemistry, and their therapeutic activities have been tested, such as spasmolytic, anti-diarrhea, antifungal, antipyretic, antioxidant, anticancer, anti-HIV, antibacterial, antiviral, anti-inflammatory, anticonvulsant (Ersöz et al., 2002; Chou et al., 2003; Lin et al., 2009; Shang et al., 2010).

In despite of the great interest in species of *Scutellaria*, we did not find any studies about plant anatomy which have been carried out in Brazilian species.

Such studies are also rare on other continents where these species occur. The literature shows only general characterization, as the brief anatomical description of the leaves and stems of *Scutellaria pinnatifida* A. Hamit subsp. *pichleri* occurring in Iran (Hatamneia et al., 2008), and a morpho-anatomical study of the vegetative and reproductive organs of *Scutellaria orientalis* L. subsp. *bicolor* (Hochst.) Edmund, subsp *santolinoides* (Hausskn ex Bornm) (Ozdemir & Altan, 2005) and subsp. *pinnatifida* Edmondtson (Candan & Çali, 2012), occurring in Turkey. The mai characteristics described for *Scutellaria* are the presence of specialized cells that have "scutellarin" on the abaxial epidermis; diferents types of glandular trichomes which can be a large single-cell, two-cells or more than sixteen cells on the head and they can be penduculate or not. These characteristics are emphatazing as important to taxonomy and were used in identification keys to distinguish this genus among the Lamiaceae (Metcalfe & Chalk, 1957).

In the Brazil, *Scutellaria agrestis* were registrated only in the North region in the Pará, Acre and Amazonas state (Harley et al., 2013). This species is cultivated and used by the indigenous Tikunas communities of Colombia, Peru and Brazil for medicinal purposes, in the treatment of stomach disorders and diarrhea. This traditional use, alongside the increased domestication of this species over time, was passed on to other descendant communities (Alonso, 1990). Nowadays, this species, commonly known as Purple Shamrock, is cultivated by populations of riverside communities of the Amazon region, in the Solimões River flood plain, and has been used as a household remedy for the treatment of earache, fever, diarrhea, high blood pressure and other diseases specific of the nosology of these communities (Cassino, 2010).

In gardens and ecological parks in the city of Manaus (State of Amazonas, Brazil), *Scutellaria agrestis* is either incorrectly identified (*Hipytis* sp.) or, more often, it is not identified at all, and is recorded only by its common name. This fact is a cause for concern, as it can lead health problems for the population that uses this plant for medicinal purposes; given that morphologically similar plants may actually be different species, it is very common for the same species to receive different common names, or for same common name to be used to denominate different plant species (Borrás, 2003).

Considering that anatomical characteristics are important taxonomic parameters for the certification and quality control of medicinal plants, an investigation of the structure of the vegetative organs of the species with therapeutic activities is necessary. These investigations are even more important for newly-identified species, to determine their anatomy, as in the case of *Scutellaria agrestis.* This paper therefore provides an anatomical and histochemical characterization (complementary analysis) of the vegetative organs of *Scutellaria agrestis* A. St.-Hill ex Benth.

Materials and Methods

For the anatomical study, five individual cultivated samples of *Scutellaria agrestis* A. St.-Hil. ex Benth., Lamiaceae, were collected, from riverside communities of Nossa Senhora das Graças, located at Costa do Pesqueiro (3º20'S, 60º36'W), and Nossa Senhora de Nazaré, located at Costa do Paratari (3º34'S, 60º55'W), in flood plain areas, in the rural zone of the municipality of Manacapuru/State of Amazonas, on the banks of the Solimões River.

Voucher specimens were deposited at the Herbarium of the National Amazon Research Institute (INPA232920), and the taxonomic identity was confirmed by specialist Prof. Dr. Alan Paton (Kew Gardens, England). Samples of the root, stem and leaf (petiole and leaf blades, young and adult) were collected from these individual samples or populations, placed in neutral buffered formalin (NBT:buffer phosphate, formalin, 9:1 v/v), kept under vacuum in a desiccator for 48 h and then preserved in ethanol 70% (Kraus & Arduin, 1997).

For the clearing, entire leaves were placed in aqueous sodium hydroxide 10% for five days. The solution was replaced every 12 h and rinsed in running water, followed by staining with safranin 1% in ethyl alcohol 50% (Johansen, 1940).

From the material fixed, samples of the root were collected (60 mm above the radicular apex), of the stem (100 mm above the stem apex) and of the leaf (fragments of its base, middle area and apex, from both the leaf blade and petiole), then embedded in methacrylate (Historesin, Leica) and sectioned in a rotary microtome (RM 2155, Leica Microsystems Inc., Deerfield, USA).The sections (transverse and longitudinal, 6-8 μm thick) were stained with toluidine blue, pH 4.0 (O'Brien &Mccully, 1981) for structural characterization.The slides were mounted with synthetic resin (Permount-Fisher). Part of the histological sections were treated with PAS (periodic acid Schiff) staining for general polysaccharides (Maia, 1979).

Histochemical tests were performed, using transverse sections of the leaf blade, petiole, stem, and roots from fresh samples.The remaining sections, which were not stained, were photographed, to record the original color of the analyzed tissues (white). Control sections were prepared simultaneously, according to the methodology described in the protocols. The reagents used included: ruthenium red (Johansen, 1940) for acid polysaccharides/mucilage; lugol for starch (Jensen, 1962); 5% tannic acid/3% iron (III) chloride for mucilage (Pizzolato & Lillie, 1973); 10% potassium dichromate for phenolic compounds (Gabe, 1968); ammonia solution for

anthocyanins (Johansen, 1940); vanillin hydrochloridric acid for tannins (Mace & Howell, 1974); phloroglucinol for lignins (Johansen, 1940); and sudan IV for total lipids (Brundett el al.,1991).

The images were obtained on a photonic microscope (model AX 70 TRF, Olympus Optical, Tokyo, Japan), with U-PHOTO system coupled to a video camera and computer equipped with image analyzer (Image Pro-Plus).

To describe the superficial characters of the leaf blade, part of the samples were subdivided into small pieces (100 mm^2), dehydrated in ethyl alcohol series, and dried to critical point (Bozzola & Russel, 1992). After fixing the samples in the supports, gold coating was applied with Sputter Coater device (model FDU 010, Bal-Tec, Balzers, Germany). A scanning electron microscope was used to capture the images (model LEO 1430 VP, Zeiss, Cambridge, England).

The arrangement of the vascular system of the petiole were classified according to Howard (1979); the arrangement of veins was classified according to Hickey (1979); the type of stomata in the leaf blade followed Wilkinson's classification (1979); and the trichomes were analyzed according to Theobald et al. (1979).

Results

Scutellaria agrestis is a prostrateplant, with straight branches reaching approximately 450 mm in height, branched; pubescente, with hairs throughout the aerial part of the plant. The stem is square, and purplish in color (Figure 1A).The phyllotaxy follows an opposite and crossing pattern (Figure 1B). The petiole measures around 6-10 mm in length and is purple in color. The leaf blade is simple and bicolor, with a green adaxial side and a purple abaxial side; it presents prominent primary and secondary vein formation (Figure 1B) and membranaceous texture. The leaf blade is oval to cordiform; the apex is sharpened and slightly retuse at the end when observed in stereoscope; the base is cordate, with a convexly indented and slightly crenate margin (Figures 1B; 2A-D). The flowers are labiate, ranging from white to violent, arranged in a terminal inflorescence, forming a type of crest (Figures 1A, 1C).

The pattern of vein formation of the leaf blade of *S. agrestis* is of the eucamptodrome and basal acrodome types, with randomized areolation, *i.e.* without any preferential orientation pattern, whether in the format of the areolae or in the venule terminations (Figures 2A-D).

Several tector trichomes with preserved cytoplasmatic content and different sizes are distributed throughout the leaf surface, and may be comprised of single or multiple cells (2-7 cells) and uniseriate; they are generally slightly curved at the apex and rarely straight, and are coated with a granulous cuticle (Figures 3A-D). Long tector trichomes with a higher number of cells are observed on the adaxial side, covering the entire surface of the leaf blade, while small trichomes are concentrated at the margins, covering the veins on both sides (Figures 3A-B). Capitate and peltate multiple cell glandular trichomes are observed on both sides of the leaf blade, with a larger number of peltated trichomes on the abaxial side (Figure 3 D-E). The capitate trichome has a short pedicel and approximately four cells composing the head (Figures 3E-F). Meanwhile, the peltate trichomes are usually located in a slight depression, with a short pedicel and multiple cell head composed of 5-12 cells, and a cuticle that distends with the accumulation of synthesized compound in the subcuticular space (Figures 3E, 3G).

The leaf is amphi-hypostomatic, with diacytic stomata (Figures 3H-I), and is slightly higher than the

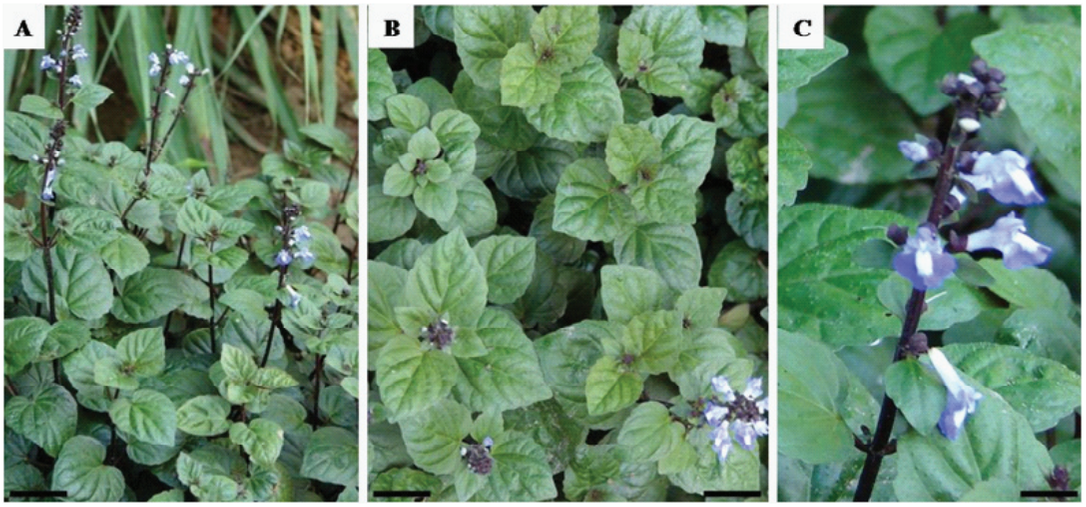

Figure 1. Plants of *Scutellaria agrestis*. A. general aspect; B. detail of the phyllotaxy; C. detail of the inflorescence (Bar: A. 2 mm; B. 1 mm; C. 0.6 mm).

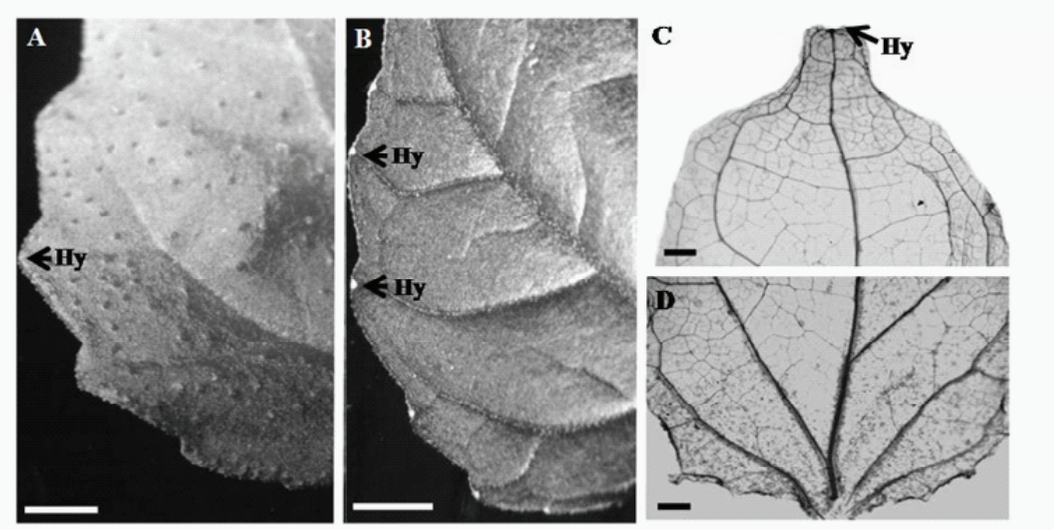

Figure 2. Leaf blade of *Scutellaria agrestis*. A. adaxial surface; B. abaxial surface, showing hydathodes in the apex of the marginal indentations; C. apex of the diaphonized leaf blade showing the apical hyatode; D. base of the diaphonized leaf blade (Hy: hydathode. Bar: A-B. 0,4 mm; C-D. 230 µm).

other epidermal cells (Figure 3J).

In frontal view, the leaf blade has an epidermis with a sinuous anticlinal wall and convex to conical periclinal wall, on both sides (Figures 3H-I). The epidermis is uniseriate, with a smooth external periclinal wall, which is thicker than the inner wall, covered by a thin, lipidic cuticle (Figures 3J, 3K).

The epidermal cells are wide, juxtaposed, tabular, and of different sizes on both sides (Figure 3J). On the abaxial side, the cells store tannins and anthocyanins in the vacuoles (Figures 3L-N). The pigment is red (Figure 3L), when observed in a freshly-cut section, and changes color when the pH is changed from acid to base using an ammonium solution, which results in a bluish color (Figure 3N). The mesophyll is heterogeneous, dorsiventral, with palisade parenchyma and around three layers of lacunous parenchyma (brachyform, when observed in frontal view, as in Figure 3O), with many intercellular spaces.

Hydathodes were observed at the apex of the mid veins and in the indentations of the edge of the leaf blade, where they are located, specifically, in valleculae (Figures 2A-D; Figures 4A-B). These structures are totally different from the initial stage of development of the leaf blade, and are present until the adult phase (Figures 4C-D).Their vascularization is formed exclusively by xylem, and they have terminal elements with helical thickening (Figure 4E). The subepidermal epithem consists of densely stained juxtaposed parenchyma cells, surrounded by an open sheath. The chambers of the aqueous pores are two to three times larger than the substomatal chambers (Figures 4C-D). In frontal view, about six aqueous pores are observed in each hydathode at the margin, and approximately twelve pores are observed in the apical hydathode, with the ostiole always open in all the hydathodes (Figure 4F). The apical hydathode is larger than the hydathodes marginal ones, with a larger number of aqueous pores and parenchyma cells that compose the epithem (Figure 4D).

The median veins are flat and convex shape, with epidermal cells of smaller sizes in the region of the leaf blade. Subjacent to the epidermis on the adaxial side there are two to four layers of lamellar collenchyma and only one layer of annular collenchyma on the abaxial side. The vascular tissue is collateral and is arranged in three bundles, which are connected in the form of open arches, surrounded by cell parenchyma (Figure 5A).

In cross-section, the petiole shows a concave-convex contour on the adaxial side and a convex contour on the abaxial side, forming two ribs (Figure 5B). The epidermis is uniseriate, with thin wall cells, storing anthocyanins in the vacuole, as described for the lamina, with intense red color when observed in microscope (Figure 5C), alluding to the purplish color observed by the naked eye, on both the abaxial side of the leaf blade and on the stem (Figures 1A-D). Tector and glandular trichomes, similar to those described for the leaf blade, can be seen on the epidermal surface (Figures 5B-E).

The cortex is composed of discontinuous annular collenchyma, with two layers of cells in the rib region and one layer in the vallecula and in the convex contour. Below the collenchyma there are about three to four layers of homogeneous chlorophyllian parenchyma with many intercellular spaces (Figure 5D).

The vascular system is collateral, arranged in three vascular bundles - a central, larger bundle, with open arch, two auxiliary, central, larger, bundles, and other two auxiliary, smaller, cylindrical bundles (Figure 5B).

In cross-section, the stem is square in shape, with

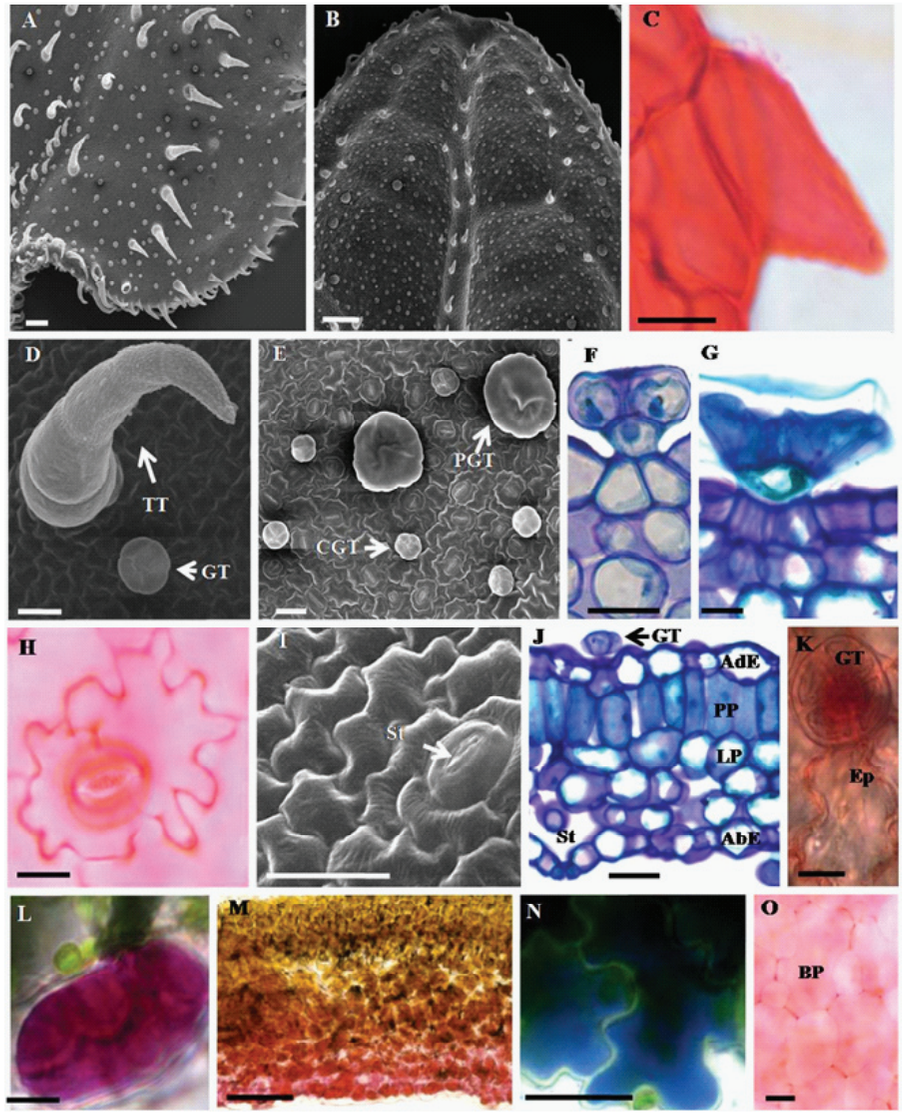

Figure 3. Structures of the leaf blade of *Scutellaria agrestis*. A-B. adaxial and abaxial surface in scanning electron microscopy (SEM); C-D. unicellular and multicellular tector trichome (SEM); E. trichomes in the abaxial surface in SEM; F-G. capitate and peltate trichome in cross-section; H-I. diacytic stomata on the abaxial and adaxial surface (SEM); J. mesophyll in cross-section; K. lipidic epidermal cuticle (sudan IV); L-N. abaxial epidermis storing antocyanin (fresh section), tannin (chlorohydric vanillin) and anthocyanin (ammonium solution), respectively; O. lacunous brachyform parenchyma. (AdE: adaxial epidermis; AbE: abaxial epidermis; Ep: epidermis; St: stomata; BP: brachyform parenchyma; LP: lacunous parenchyma; PP: pallisade parenchyma; GT: glandular trichome; CGT: capitate glandular trichome; PGT: peltate glandular trichome; TT: tector trichome. Bar: A. 100 µm; B, D. 200 µm; C, F, H, L, O. 10 µm; E, I. 20 µm; G. 110 µm; J, N. 24 µm; K. 12 µm; M. 35 µm).

prominent vertices showing ribs (Figure 5F). The epidermis consists of a layer of polygonal cells that are coated with a thin lipid cuticle (Figure 5G). In the epidermis, cells storing anthocyanins, stomata, tector and glandular trichomes can be seen, similar to that described for the petiole.

In the outer portion of the cortex, uniseriate lamellar collenchyma are observed, between the vertices and multiple stratifications (two or three layers) at the vertices; below the collenchyma, three to four layers of chlorophyllian parenchyma cells are arranged with a large number of intercellular spaces (Figure 5H). The endodermis is evident, with large cells, which are stained red by sudan IV (Figures 5G, 5I).

The vascular system is continuous and collateral, with active fasciculation at the region of the vertices and interfasciculation at the region of the valleculae (Figures 5F, 5H). Groups of fibers are appended to the phloem (Figures 5H-I).The pith is parenchymatous, with large cells and many intercellular spaces (Figure 5F).

The root is covered by a uniseriate epidermis with common, tabular cells and root hairs, forming a tangle (Figure 6A). In the outermost layer of the cortex,

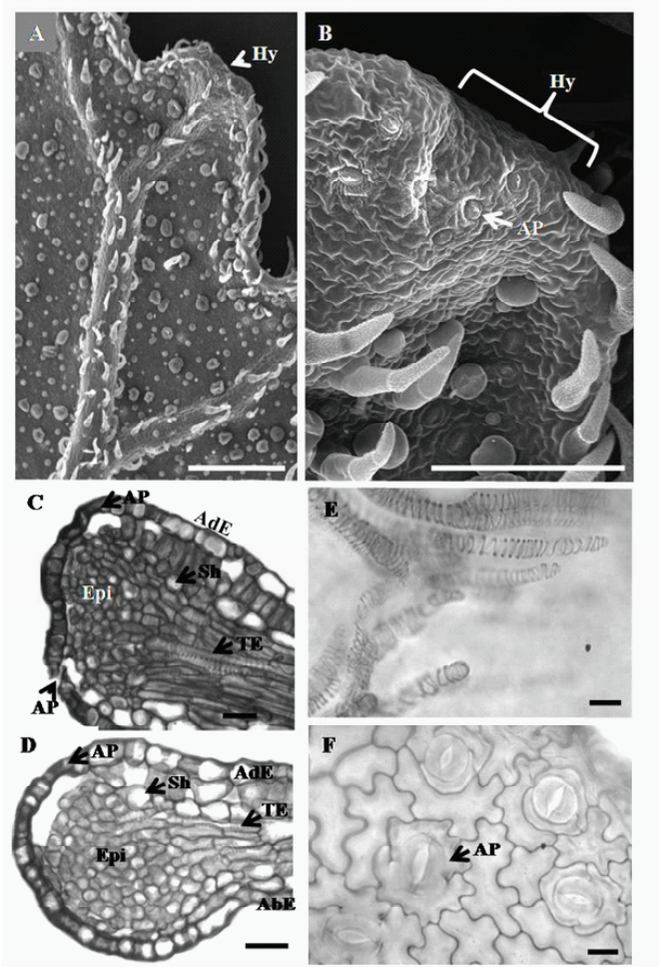

Figure 4. Hydathodes in the leaf blad of *Scutellaria agrestis.* (A-C, E-F, hydathode in the indentationsof the leaf margin and D, hydathode in the apex of the leaf blade). A. general view of the abaxial surface in SEM; B. aqueous pores; C. hydathode in the young limb in longitudinal section; D. hydathode in the adult limb in longitudinal section; E. helicoid tracheal elements in the hydathode; F. aqueous pores in the hydathode. (AbE: abaxial epidermis; AdE: adaxial epidermis; AP: aqueous pore; Epi: epithem; Hy: hydathode; Sh: sheath; TE: tracheal element. Bar:A. 300 µm; B. 100 µm; C, D. 24 µm; E, F. 10 µm).

a uniseriate exodermis can be seen, with large, cubical cells and accumulated anthocyanins (Figures 6A-B). The cortex is homogeneous, consisting of parenchymal tissue with several intercellular spaces (Figure 6A). The endodermis is evident, with the cell walls stained red by sudan IV (Figures 6C-D, 6I). The cortical cells reacted positively to potassium dichromate, revealing the presence of phenolic compounds; the reaction was more intense on the endodermis (Figure 6E). In the same region, the presence of mucilage was observed (Figure 6F). Negative results were obtained for the tannin and reserve compound tests, such as general polysaccharides, starch and lipids.

The sudan IV test revealed several varied microorganisms (protists, including some diatoms), detected by red staining of their cell membranes. These organisms probably occur in the soil, and may be present on the surface of the root hairs and in all the intercellular spaces of the root (Figures 6G-I). The root hairs are present in abundance and vary in size - from short to long.

The vascular cylinder is delimited by one a layer of voluminous pericyclecells (Figure 6C). The vascular structure is pentarch, arranged in five groups of protoxylem with metaxylem in the center, alternating with phloem, forming a structure that is typically protostelic (Figure 6C).

Discussion

Representatives of Lamiaceae can occur in various habitats. Many species present characteristics of arid environments, *i.e.* xeromorphic characters. The smallest group of the family, including the genera *Dysophylla, Mentha* and *Scutellaria*, is characteristic of moist environments (Metcalfe & Chalk, 1957).

Scutellaria agrestis A. St.-Hil. ex Benth., presents both simple uni and multicellular tector trichomes and

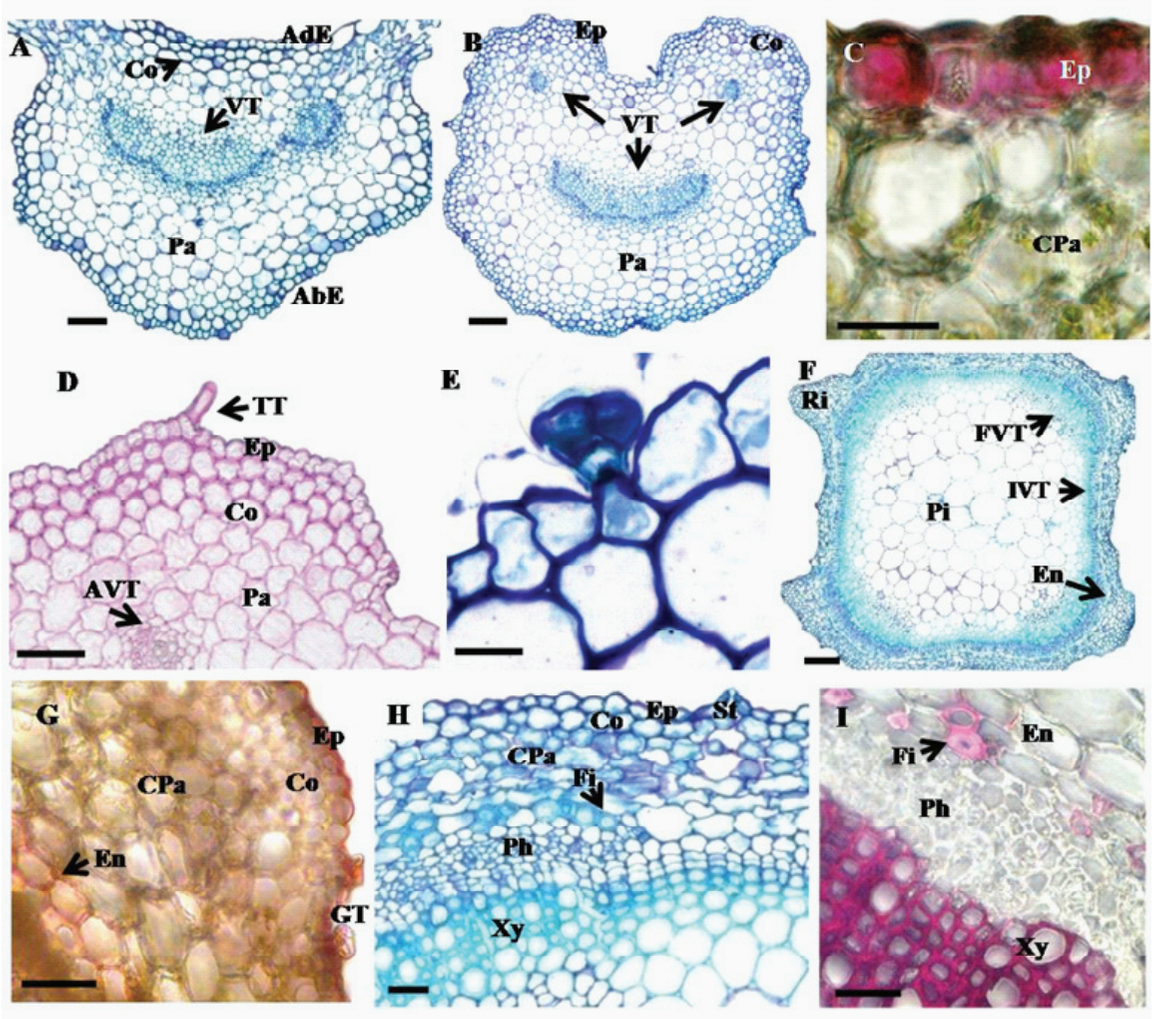

Figure 5. Structures of the median nervure, petiole and stem of *Scutellaria agrestis.* (Cross sections. B-E, petiole and F-I, stem). A. median nervure; B. general appearance; C. cells of the epidermis storing anthocyanin; D. collenchyma (PAS) in the rib region; E. capitate trichome; F. general appearance; G. epidermis and endoderm evidenced by the sudan IV; H. cortex and vascular tissue; I. phloematic fibers and cells of the xylem (phloroglucinol). (AbE: abaxial epidermis; AdE: adaxial epidermis; AVT: accessory vascular tissue; Co: collenchyma; CPa: chlorophyllian parenchyma; Ep: epidermis; En: endodermis; Fi: fiber; FVT: fasciculated vascular tissue; IVT: interfasciculated vascular tissue; GT: glandular trichome; Pa: parenchyma; Ph: phloem; Pi: pith; Ri: rib; St: stomata; TT: tector trichome;VT: vascular tissue; Xy: xylem. Bar: A. 70 µm; B. 110 µm; C. 25 µm; D. 60 µm; E. 15 µm; F. 150 µm; G. 40 µm; H. 30 µm; I. 24 µm).

glandular trichomes of the capitate and peltate types.

These epidermal structures are common characters for Lamiaceae, with tector trichomes varying from simple to branched, and glandular trichomes of the peltate and/or capitate type, generally varying in the number of cells that comprise the secretory head (Metcalfe & Chalk, 1957; Werkeret al., 1993). Information on the combination between quantity and type, orientation and size of the trichomes is of great taxonomic value for the Lamiaceae family, particularly for the *Scutellaria* genus (Metcalfe & Chalk, 1957; Werkeret al., 1993; Ascensão et al., 1995; 1997; Kaya et al., 2007; Pool, 2006; Giuliani & Bini, 2008). These structures have been recorded in various species of *Mentha* L. (Bozani et al., 2007), thirteen species of *Hypenia* (Mart. ex Benth.) R. Harley (Faria, 2008), as well as in other representatives of Lamiaceae (Metcalfe & Chalk, 1957; Toledo et al., 2004; Duarte & Lopes, 2005; 2007; Basílio et al., 2006).

In *Scutellaria*, the presence of secretory trichomes of the capitate type is reported, with a generally long pedicele, unicellular and bicellular secretory head, and in rare cases, the occurrence of secretory trichomes with sixteen cells comprising the

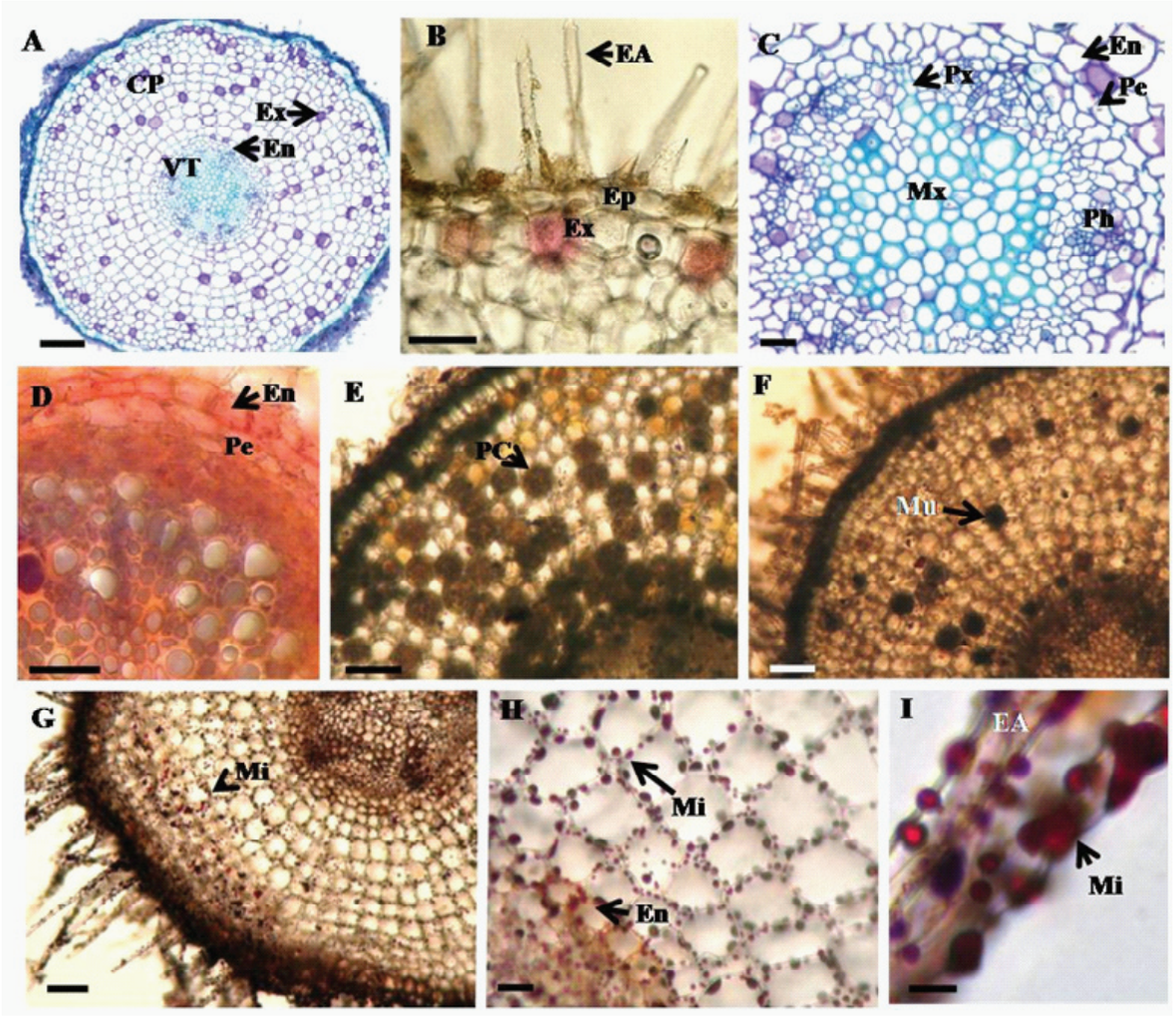

Figure 6. Structure of the root of Scutellaria agrestis. (crosssections). A. general appearance; B. exodermstoring anthocyanin; C. endodermis and vascular tissue; D. endodermis (sudan IV); E. cells with phenolic compound (potassium dichromate); F. cells with mucilage (tannic acid/iron (III) chloride); G. general appearance of the root with endophytic microorganisms stained red (sudan IV); H. endophytic microorganisms in the cortical region (sudan IV); I. epiphytic microorganisms adhering to the epidermal appendices (sudan IV). (CP: cortical parenchyma; EA: epidermal appendix; En: endodermis; Ep: epidermis; Ex, exodermis; Mi: microorganism; Mu: mucilage; Mx: metaxylem; PC: phenolic compound; Ph: phloem; Pe: pericycle; Px: protoxylem; VT: vascular tissue. Bar: A. 110µm; B. 20 µm; C. 24 µm; D. 40 µm; E-G. 130 µm; H. 35 µm; I. 15 µm).

head of the peltate type (Metcalfe & Chalk, 1957). It is emphasized that the characterization of these trichomes is of primary importance, as there are few reports in the anatomical literature for the genus, such as the presence of three subtypes of trichomes of the capitate type for *S. orientalis* L. subsp. *bicolor* Edmund and subsp. *santolinoides* (Hausskn ex Bornm) (Ozdemir & Altan, 2005) and two subtypes for the subsp. *pinnatifida* Edmondtson (Candan & Çali, 2012). The presence of secretory trichomes of the peltate type for *S. orientalis* L. subsp. *bicolor* and subsp. *pinnatifida* (Ozdemir&Altan, 2005; Candan & Çali, 2012, respectively) and with a cell head composed of sixteencells *S. gallericulata* (Giuliani & Bini, 2008). Reported the presence of the trichomes capitates (uni or multi cellular) and peltate in petiole for *S. salviifolia* Benth. (Akçin et al., 2011) and in leaf blade and calyx in *S. altissima* L. (Thaler et al., 1992). Even, the presence of secretory trichomes in leaf blade for *S. lateriflora* (Gafner et al., 2003). Also, the absence of secretory trichomes in *S. pinnatifida* subsp. *pichleri* (Hatamneiaet al., 2008).

An amphi-hypostomatic leaf, as observed in *S. agrestis*, was also described in *Cunila microcephala* Benth. (Toledo et al., 2004) and in the thirteen species of

Hypenia (Faria, 2008). The stomata in Lamiaceae may occur in one or both sides of the leaf blade (Metcalfe & Chalk, 1957). For example amphi-stomatic leaves are observed in *S. orientalis* L. subsp. *pinnatifida* (Candan & Çali, 2012), in various species of *Mentha*, in *Hyptis suaveolens* L. (Poit) (Bozaniet al., 2007; Basílio et al., 2006, respectively) or hypostomatic in *S. orientalis* L. subsp. *bicolor* and subsp. *santolinoides* (Ozdemir & Altan, 2005), in *S. altissima* (Thaler et al., 1992), in *Hyptis pectinata* (L.) Poit., *Plectranthus neochilus* Schltr. and, *Leonurus sibiricus* L. (Basílio et al., 2006; Duarte & Lopes, 2007; Duarte & Lopes, 2005, respectively). The variation observed indicates that this characteristic may be useful for different iating genera and/or species of Lamiaceae.

The epidermal layer of the aerial vegetative organs of *S. agrestis* is comprised of anthocyanin-storing cells, a substance responsible for the purple colour of the lower side of the leaf blade, petiole and stem when seen with the naked eye. The occurrence of special epidermal cells for the genus *Scutellaria* was mentioned by Metcalfe & Chalk (1957), and it is also recorded on the stem of *Mentha spicatus* (Bozani et al., 2007), and on the adaxial side of the leaf blade of *Coleus blumei* Benth. (Fisher, 1985). These pigments are hydrophilic and non-toxic, and are responsible for some of the colors of fruits, vegetables, flowers and other vegetal tissues. Anthocyanins have different colors that may vary according to the pH. When the pH is lower than 2, the anthocyanins exist mainly in the form of red-colored flavyliumcation; if the pH is changed to 6, this compound will be converted into quinonoid bases, resulting in a purple coloration (Bakowska et al., 2003).

Among the various known functions of anthocyanins present in the reproductive organs, they act as attractants for pollinators or spread seeds. Although little is known about their role in vegetative organs, their role in chemical defence of the plant against viruses, fungi and bacteria, protection against UV and hormonal activity regulators is emphasized (Buchanan et al., 2000; Castro et al., 2004; Rausher, 2006). In *S. agrestis* the presence of these pigments may be related to protection against UV rays, considering that they grow mainly in sunny environments, and the herbaceous habit of the plant. It is probably also related to the defence of the plant against microbial colonization (Harborne & Williams, 2000).

Marginal hydathodes are present in the leaf blade of *S. agrestis.* There are few records of hydathodes for representatives of Lamiaceae and no description for *Scutellaria*. Among the numerous anatomical works already carried out on the family, their occurrence in *Coleus blumei* (Fisher, 1985) and in four representatives of *Hypenia* (*H. glauca, H. durifolia, H. reticulate* and *H. crispata*) is emphasized, among the thirteen species investigated (Faria, 2008).

The hydathodes are formed very early in the process of development of the leaf blade and in some cases, they cease to function even before the organ reaches maturity, as pointed out for *Hygrophila polysperma* (Roxb.) T. Anderson, Acanthaceae (Reams, 1953). In *Ficus formosana* Maxim., Moraceae, the functioning of the active laminar hydathode is reported only in the final stage of maturation of the limb (Chen & Chen, 2006). In *S. agrestis,* the hydathode is formed in young limbs. Meanwhile, further physiological investigation is needed, since in maturity, the aqueous pores are all open and unobstructed, denoting a possible functioning from a structural point of view, even though the ability to control the process of opening the aqueous pores is less than zero, due to thickening of the cuticle in maturity (Reams, 1953; Evert, 2006).

The function of the hydathode is to secrete a liquid of variable composition, from pure water to organic and inorganic solutes, through dripping from the aqueous pores, a phenomenon known as guttation (Fahn, 1979; Evert, 2006). This process occurs when, under the right conditions (high soil moisture and air humidity), the excess water generated by the positive balance of the entry of water to the root and reduction or absence of leaf transpiration is eliminated by the hydathodes (Fahn, 1979). The appropriate environmental conditions for guttation to occur are usual in the North of Brazil, particularly in the Amazon, where the species in question grows.

The epithem hydathode (Fahn, 1979) is the type observed in *S. agrestis.* The epithem tissue is formed by a parenchyma with abundant organelles, including the Golgi complex, mitochondria, endoplasmatic reticulum, peroxisomes, ribosomes, plastids (except for chloroplasts) and many small vacuoles within the cytoplasm (Fahn, 1979; Evert, 2006; Chen & Chen, 2006). Studies indicate that various genes are expressed in hydathodes, such as GD1 (glutamine dumper), which acts in the regulation and exportation of amino acids and PHO1, which acts in the absorption and acquisition of phosphate. This information suggests that these genes exert an important function of restoring nutrients during guttation and possibly in the defence of the plant, since the hydathodes are reported as a point of entry for pathogenic agents such as epiphytic bacteria (Fahn, 1979; Hugouvieux al., 1998; Pilot et al., 2004; Wang et al., 2004; Evert, 2006; Chen & Chen, 2006).

In *S. agrestis organs*, we observed tissues with abundant intercellular spaces were recorded in all the organs analyzed. This observation is reported for various species of *Ocimum, Salvia, Hypenia*, among others belonging to the Lamiaceae (Metcalfe & Chalk, 1957; Farias, 2008). The presence of these intercellular spaces is seen not only in vegetative organs of the representatives of Lamiaceae, but also in reproductive organs, as in

the fruit of *Scutellaria minor* Hudson, specifically in the structure of the mesocarp (Mosqueiro et al., 2002). The author links the presence of these air spaces to the type of dispersion (nautocoria) of the fruits of *S. mino*r; these spaces enable the fruit to float at the moment of dispersion, as this species grows on riverbanks, where the surrounding vegetation is subject to periodic flooding (Mosqueiro et al., 2002). In the leaf blade of *S. agrestis*, a lacunous parenchyma was recorded, with a characteristic of brachyform cells, *i.e.* numerous intercellular spaces. This characteristic may be related to the natural environment of this species, whether of this genus, since the distribution center of *Scutellaria* normally occurs in moist, flooded environments as already described for *S. mino*r, or other species of the genus (Alonso, 1990; Mosqueiro et al., 2002; Shang et al., 2010).

The mechanical support tissue seen in *S. agrestis* is the collenchyma, which are present in the leaf blade, petiole and stem vertices. Even, observed in the petiole of *S. salviifolia* (Akçin et al., 2011) and in the stem of *S. lateriflora* (Gafner et al., 2003). These support cells are characters that are normally related to the herbaceous habit and to the fact that the plant grows in moist environments (Evert, 2006), as is the case with the species in question. Meanwhile, for species that grow in dry environments, the sclerenchyma is the predominant support tissue, as recorded for species of *Hypenia* (Faria, 2008).

A square stem with prominent vertices and visible endoderm, as described in this work for *S. agrestis* and also for other species of this genus, such as *Scutellaria orientalis* L. subsp. *pinnatifida* (Candan & Çali, 2012), subsp. *bicolor* and subsp. *santolinoides* (Ozdemir & Altan, 2005) are diagnostic characteristics present in representatives of Lamiaceae, according to Metcalfe & Chalk (1957).

In the root of *Scutellaria agrestis*, substances have been described such as phenolic compounds and mucilages in the cortical cells. Some microorganisms have also been evidenced in the cortex. In the rhizosphere of the plants, the microfauna is diverse, due to the high quantity of nutrients secreted and released by the roots (Willadino et al., 2005). These nutrients also constitute efficient attractants for opportunistic chemoorganotrophic microorganisms (Salaet al., 2000; Willadino et al., 2005). The presence of these microorganisms may be related to the numerous intercellular spaces in the cortex, which can serve as a host and the compounds secreted in the roots, which serve as attractants. Further research is therefore recommended, on the identification and probable associations between the species in question and the agents detected.

Conclusion

Anatomical characters of diagnostic value in *Scutellaria agrestis* were recorded in the present study, in particular, the presence of an epithem hydathode, described for the first time for the genus. Furthermore, ordinary characters of the Lamiaceae family and the *Scutellaria* genus were found, such as multicellular tector trichomes and glands of the capitate and pelatate types, diacytic stomata, numerous intercellular spaces, and a square stem with evident endodermis. This information is important, as it helps in the identification of the species and contributes to its quality control, given that the plant in question is used for medicinal purposes.

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Authors constributions

MFC contributed in collecting plant material and critical reading of the manuscript. MCO contributed in herbarium confection. NM and MV contributed to running the laboratory work. MT contributed in getting papers and reading of the manuscript. All the authors have read the final manuscript and approved the submission.

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***Correspondence**

Renata Maria Strozi Alves Meira
Plant Anatomy Laboratory, Federal University of Viçosa
Avenida P. H. Rolfs, s/n Campus Center, 36560-000 Viçosa-MG, Brazil
rmeira@ufv.br
Tel. 55 31 3899 2584